HIGH SENSITIVITY C- REACTIVE PROTEIN AS A PREDICTOR OF ATHEROSCLEROTIC CORONARY ARTERY DISEASE IN DIABETES MELLITUS

Dissertation

M.D. (BRANCH XIII)

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CERTIFICATE

This is to certify that this dissertation on **"HIGH SENSITIVITY C- REACTIVE PROTEIN AS A PREDICTOR OF ATHEROSCLEROTIC CORONARY ARTERY DISEASE IN DIABETES MELLITUS**" is a work done by **Dr.B.Gayathri**, under my guidance during the period 2005 – 2008. This has been submitted in partial fulfillment of the award of M.D Degree in Biochemistry (Branch – XIII) by the Tamilnadu Dr. M.G.R. Medical University, Chennai.

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DECLARATION

I, **Dr.B.Gayathri,** solemnly declare that dissertation titled, **"HIGH SENSITIVITY C- REACTIVE PROTEIN AS A PREDICTOR OF ATHEROSCLEROTIC CORONARY ARTERY DISEASE IN DIABETES MELLITUS**" is a bonafide work done by me at Government Stanley Medical College and Hospital during 2005 - 2008 under the supervision and guidance of **Prof.Dr.P.Jayanthi M.D,** Professor and Head, Department of Biochemistry, Stanley Medical College, Chennai.

The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, towards the partial fulfillment of requirement for the award of M.D. Degree (Branch XIII) in Biochemistry.

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CONTENTS

ABBREVIATIONS

hs –CRP	- High sensitivity C- reactive protein
LDL	- Low density lipoprotein
HDL	- High density lipoprotein
VLDL	- Very Low density lipoprotein
TGL	- Triacylglycerol
TC	- Total Cholesterol
VCAM	- Vascular cell adhesion Molecule
ICAM	- Intra cellular Adhesion Molecule
PECAM	- Platelet Endothelial Cell Adhesion Molecule
MCP 1	- Monocyte chemotactic protein 1
IL 1	- Interleukin 1
IL 6	- Interleukin 6
TNF α	- Tumour necrosis factor alpha
PDGF	- Platelet derived growth factor
TGF α	- Transforming growth factor alpha
SAA	- Serum Amyloid A
AHA/ CDC	- American Heart Association/ Center for Disease Control
AGE	- Advanced Glycated End Products
RAGE	- Receptor for Advanced Glycated End Products

EDTA - Ethylene Diamine Tetra Acetic Acid

INTRODUCTION

Diabetes mellitus is characterized by increased circulating plasma glucose concentrations associated with abnormal metabolism of carbohydrate, protein, fat and a variety of micro vascular and macro vascular complications. The prevalence of both type 1 and type 2 diabetes has risen dramatically over the past two decades and is expected to rise phenomenally in future due to decreased physical activity and obesity. It is predicted that type 2 diabetes will be increased to pandemic proportions in a few decades in Indian population. Cardiovascular disease is one of the leading cause of mortality and morbidity in patients with diabetes. It results from the sequlae of atherosclerotic coronary artery disease and the most common manifestations are acute myocardial infarction, angina, heart failure and sudden death.

Atherosclerosis, the underlying cause of coronary artery disease starts early in life and progresses slowly usually for decades. It is not simply a disease of lipid deposition, but of an endothelial injury leading to endothelial dysfunction and local inflammatory changes which predispose to the formation of atherosclerotic plaque. These changes play a pivotal role in athero thrombotic disease progression. Diabetes is an independent risk factor for coronary artery disease and often is not associated with typical anginal symptoms, it becomes difficult to

diagnose the process of atherosclerosis at an early stage. The inflammatory changes found to be associated with atherosclerotic disease process, can be used for its identification. A well known marker of inflammation, namely C- reactive protein with the recent high sensitivity assays (hs- CRP) can detect inflammation associated with atherosclerosis at an early stage.

The present study is aimed at the evaluation of high sensitivity C- reactive protein as a marker for atherosclerosis. High sensitivity C- reactive protein assay in patients with diabetes mellitus associated with and without coronary artery disease is performed and the results are correlated with lipid profile.

AIM OF THE STUDY

The study is aimed at estimating the levels of high sensitivity C – reactive protein in diabetes mellitus associated with and without coronary artery disease.

The study is also aimed at correlating the levels of high sensitivity C – reactive protein with the biochemical markers of atherosclerosis like total cholesterol, triacyl glycerol, high density lipoprotein, low density lipoprotein, very low density lipoprotein and the use of high sensitivity C – reactive protein as a predictor of atherosclerotic coronary artery disease in diabetes mellitus.

REVIEW OF LITERATURE

Atherosclerosis is a chronic immuno inflammatory, fibro-proliferative disease, fueled by lipids, which affects primarily the intima of the medium sized and large sized arteries, resulting in intimal thickening, leading to luminal narrowing and inadequate blood supply.[1]

As the name implies, the mature atherosclerotic plaques consist of typically two main components. One is lipid rich and soft -athe're is Greek for "gruel" or "porridge" and the other is collagen rich and hard-skleros is Greek for "hard".

At the beginning of twentieth century, cardiovascular disease accounted for less than 10 percent of all the deaths worldwide. But at the beginning of twenty first century, cardio vascular disease accounts for nearly half of all the deaths in the developed world and twenty five percent in the developing world. By 2020, it is predicted that cardiovascular disease will claim twenty five million lives annually and coronary heart disease will be the number one cause of death and disability, with every one in three deaths are due to coronary heart disease.[2]

Fuster V, [3] state that atherosclerosis, with superimposed thrombosis, `athero thrombosis´ is the leading cause of death and severe disability in affluent countries, and it will soon become pandemic, due

to increased incidence of obesity, insulin resistance and type 2 diabetes mellitus.

RISK FACTORS FOR ATHEROSCLEROTIC CORONARY ARTERY DISEASE

Many prospective studies, like Framingham study established the coronary heart disease "risk" factors (i.e) factors that make the occurrence of the disease more probable.[4]

NON-MODIFIABLE	MODIFIABLE
1. Increasing age 2. Male gender 3. Family history 4. Genetic factors 5. Personality (type A)	1. Hypertension 2. Diabetes 3. Smoking 4. Obesity 5. Hyper lipidemia 6. Sedentary life style 7. High carbohydrate And trans unsaturated Fat intake.

MODIFIABLE RISK FACTORS

AGE

Age is a dominant influence. Atherosclerosis is not usually clinically evident until middle age. Between ages 40 and 60, the incidence of myocardial infarction increases five fold.

SEX

Males are more prone to atherosclerosis than females. The complications of atherosclerosis are uncommon in pre-menopausal women, unless they are predisposed by diabetes, hyper lipidemia or hypertension.

After menopause, the incidence of atherosclerosis increases due to a decrease in estrogen levels and the frequency of myocardial infarction equalizes by seventh to eighth decade of life.

FAMILY HISTORY

The occurrence of premature atherosclerotic death in the family of age group less than 55 in a male first degree relative or less than 65 years in a female first degree relative play a role in the atherosclerotic disease progression.[5]

GENETIC FACTORS

The familial predisposition to atherosclerosis and coronary ischemic disease is polygenic. It is also related to clustering of other risk factors, such as hypertension or diabetes or genetic derangements in lipoprotein metabolism.

PERSONALITY

Type A personality, also known as Type A behaviour pattern, is a set of characteristics that includes being impatient, excessively time conscious, insecure about one's status, highly competitive, hostile and aggressive, and incapable of relaxation. It was first described as an important risk factor in coronary disease in 1950's by cardiologist Meyer Friedman and his coworkers. It is estimated that Type A behaviour doubles the risk of coronary heart disease in otherwise healthy men.[6]

MODIFIABLE RISK FACTORS:

HYPER TENSION

Hypertension is a major risk factor for atherosclerosis at all ages. Both systolic and diastolic hypertension increases the risk. It is predicted that, there is a five fold risk of coronary heart disease when the blood pressure exceeds 140/90 mm Hg.

DIABETES

The incidence of myocardial infarction is twice as high in diabetics as in non- diabetics. There is also an increased risk of stroke and atherosclerotic gangrene of lower extremities in diabetic patients. Uncontrolled and long standing diabetes induces hyper cholesterolemia and it markedly increases the premature atherosclerotic disease progression.

SMOKING

Cigarette smoking accelerates atherosclerosis and promotes acute ischemic events. The mechanisms are

1. Hemodynamic stress (nicotine increases heart rate and transiently increases the blood pressure).

2. Endothelial injury and dysfunction (nitric oxide release and resultant vasodilatation are impaired).

3. Development of atherogenic lipid profile (higher levels of Low density lipoprotein, more oxidized Low density lipoprotein, lower levels of High density lipoprotein).

4. Enhanced coagulability and induces chronic inflammatory state.

5. Arrhythmogenesis.

6. Relative hypoxemia because of effects of carbon monoxide.[7]

OBESITY

Body mass index ≥30 Kg/m^2 is considered to be obesity and it plays a major role in atherosclerosis progression. Abdominal obesity rather than peripheral obesity (gluteal or subcutaneous) poses a high risk, by causing altered lipid levels and insulin resistance.

HYPERLIPIDEMIA

Hyperlipidemia is a major risk factor for atherosclerosis. Presence of hypercholesterolemia is sufficient to initiate the disease process by producing an endothelial injury in the coronary arteries. The major component of total serum cholesterol associated with increased risk is Low density lipoprotein, which serves as a vehicle for the delivery of cholesterol to peripheral tissues.

In contrast, high density lipoproteins act as “Good Cholesterol” by mobilizing the cholesterol from the developing atheroma and transport to the liver for excretion.

OTHER FACTORS

Factors associated with increased risk of atherosclerosis include a sedentary life style with lack of exercise and poor eating habits (i.e) increased carbohydrate and trans unsaturated fat intake with decrease in fiber and vegetables.

PATHOGENESIS OF ATHEROSCLEROSIS

The concept of "response to injury" hypothesis considers atherosclerosis to be a chronic, inflammatory response of the arterial wall initiated by injury to the endothelium.[8] Although the course of atherosclerotic disease progression is silent with a very long incubation period, the dreaded complications of this disease due to arterial lumen narrowing like myocardial infarction, angina, sudden cardiac death has led to focus much more attention, towards the pathogenesis of atherosclerosis.

EVOLUTION OF ARTERIAL WALL CHANGES IN ATHEROSCLEROSIS

Chronic endothelial injury is caused by factors like hyperlipidemia, circulating derivatives of cigarette smoke, homocysteine, hemo dynamic shear stress, viruses and other infectious agents. This injury causes increased endothelial permeability, enhanced

leukocyte adhesion and alterations in expression of endothelial gene products.[9]

The normal endothelium will not bind to white blood cells. In contrast, the injured endothelium, expresses adhesion molecules, that have the capacity for binding to leukocytes. It includes the vascular cell adhesion molecule -1 (VCAM -1). It binds to monocytes and T-lymphocytes and paves way to the migration of these cells to intima of the coronary artery. These cells transform into macrophages and begin to engulf the oxidized low density lipoprotein, which is formed due to the oxidative stress induced by free radicals generated in macrophages and endothelial cells in the arterial wall.[10]

Macrophages produce interleukin-1(IL-1) and tumour necrosis factor (TNF) and chemokines such as monocyte chemotactic protein -1(MCP-1) which increase the leukocyte adhesion on the endothelial cell. T-lymphocytes both CD4+ and CD8+ are also recruited to the intima and it induces cellular and humoral immune activation. T-cells cause release of inflammatory cytokines such as interferon- gamma and lymphotoxin, which in turn stimulate macrophages and smooth muscle cell replication and contribute to a dense extra cellular matrix characteristic of a more advanced atherosclerotic lesion.[11]

Several growth factors are reported to play a role in the proliferation of smooth muscle cells, which include platelet derived growth factor(PDGF), fibroblast growth factor, transforming growth factor- alpha. The smooth muscle cells also take up the modified lipids (oxidized lipids), and forms foam cells. Vascular smooth muscle cells synthesize extra cellular matrix molecules (mainly collagen) which stabilize the atherosclerotic plaque.[12]

Hence this initial event of formation of intimal plaque is an aggregation of foam cells of macrophage and smooth muscle cell origin. With progression , the atheromatous lesion is modified by smooth muscle cell synthesized collagen and extracellular matrix molecules and formation of fibrous cap with retaining of central core of lipid laden cells and fatty debris. Inflammatory events make a significant contribution in the atherosclerotic disease progression. Inflammation disrupts the fibrous cap of atheromatous lesion by over expression of matrix metallo proteinases that can break down collagen and thinning of fibrous cap, leading to plaque rupture and fatal myocardial infarction.[13,14]

DIABETES AND ATHEROSCLEROTIC CORONARY ARTERY DISEASE

Vascular diseases are the major cause of morbidity and mortality in patients with diabetes mellitus. Diabetes causes micro vascular diseases such as nephropathy, neuropathy and retinopathy and macro vascular disease such as atherosclerosis. Atherosclerosis of the coronary, cerebral and peripheral arteries account for approximately 80 percent of mortality and 75 percent of hospitalizations in persons with diabetes.[15]

The MULTIPLE RISK FACTOR INTERVENTION STUDY, has reported that men with diabetes had an absolute risk of death due to coronary artery disease three times higher than that in the non- diabetic even after adjustment for established risk factors.[16] A FINNISH EPIDEMIOLOGICAL SURVEY compared the incidence of myocardial infarction in diabetic and non- diabetic populations. In this study, patients with diabetes without prior myocardial infarction had the same level of risk for subsequent acute coronary events as the non- diabetic persons with a history of previous myocardial infarction.[17]

The prevalence of atherosclerotic coronary artery disease is lower in pre- menopausal women when compared to their male counter part. This is due to the protective action of estrogen. But the incidence of

diabetes in these women, blunts the benefit of female gender for the risk of coronary artery disease.[18]

Miettinen H et al[19], have stated that diabetes increases the risk of death due to myocardial infarction in women more than men.

PATHOPHYSIOLOGY OF DIABETIC VASCULAR DISEASE

Diabetes is characterized by metabolic abnormalities including hyperglycemia, dyslipidemia and insulin resistance that disrupt the normal arterial function and will render arteries susceptible to atherosclerosis.

PROLONGED HYPERGLYCEMIA AND PROTEIN GLYCATION

Hyperglycemic episodes, in both type1 and type 2 diabetes cause non- enzymatic glycation of macromolecules like lipids and proteins. Glycated proteins can form structures known as advanced glycated end products (AGE). Glycation of low density lipoproteins, reduces their binding to the low density lipoprotein receptor and decrease their rate of their catabolism.[20] Klen RL and Lyons TJ et al[21], have demonstrated an increased uptake of glycated low density lipoprotein by macrophages. In addition, Duel PB and Oram JF[22] have stated that glycated high density lipoprotein is cleared from the plasma at an enhanced rate, and its ability

to stimulate the cholesterol efflux from the atheroma is diminished compared to native high density lipoprotein.

Glycated proteins are immunogenic. It can produce an immune response and will contribute to the development and progression of macro vessel disease.[23] Advanced glycated low density lipoprotein, are very much increased in diabetic subjects compared to non- diabetics. The glycated end products, mediate their biological effects through the surface receptors like receptor for advanced glycated and products (RAGE). The vascular cells, produce inflammatory cytokines, which impair endothelial dependent vasodilatation and increased endothelial expression of leukocyte adhesion molecules, like E- selectin, P- selectin, Intracellular adhesion molecule-1(ICAM-1), Vascular cell adhesion molecule-1(VCAM-1), GlyCAM-1, Platelet endothelial cell adhesion molecule(PECAM) which are implicated in atherosclerosis.[24]

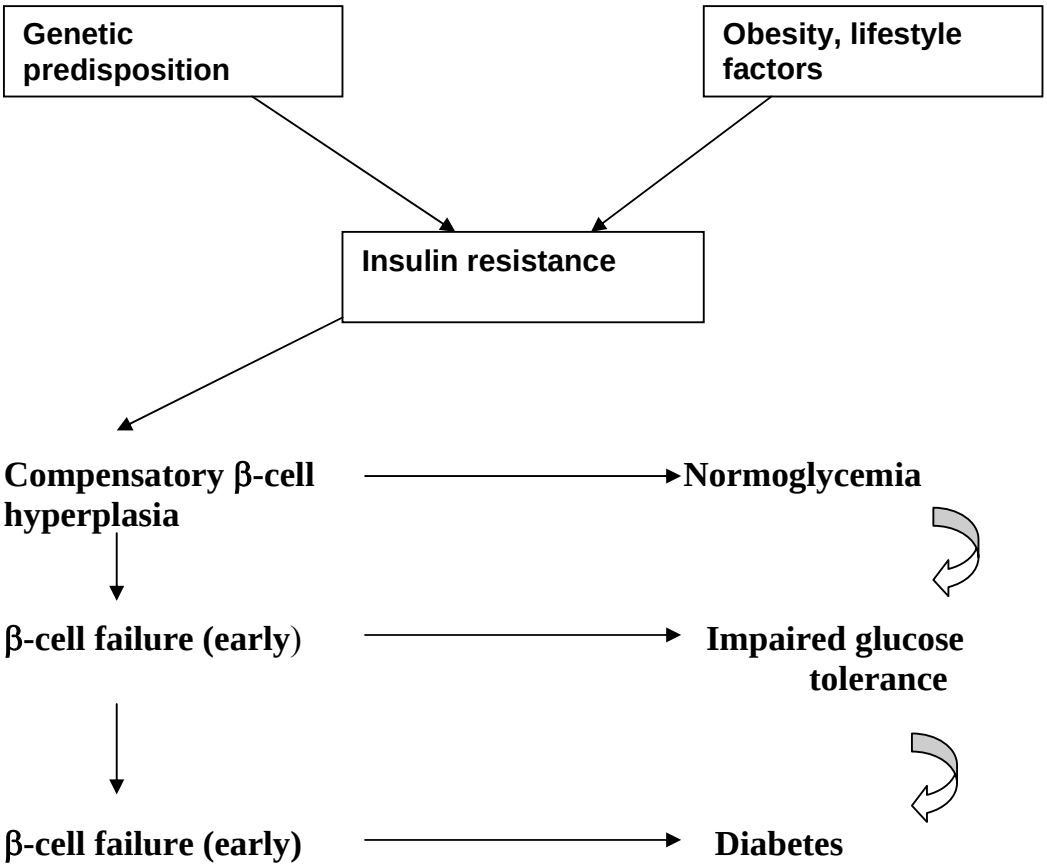
HYPERGLYCEMIA AND OXIDATIVE STRESS

Hyperglycemia increases the production of reactive oxygen species in the vascular cells through enzymatic (protein kinase C and NADPH oxidases) and non enzymatic sources (formation of advanced glycated end products) [25]. Normally, endothelium maintains the vascular homeostasis. Nitric oxide helps in this maintenance by its vasodilatory, anti-inflammatory and anti- thrombotic effects.

Hyperglycemia decreases nitric oxide production from endothelial nitric oxide synthase and it also increases its degradation via generation of reactive oxygen species. As hyperglycemia induced oxidative stress increases, the cofactor for endothelial nitric oxide synthase tetrahydrobiopterin becomes oxidized. Oxidized tetrahydrobiopterin uncouples endothelial nitric oxide synthase, and causes this enzyme to produce superoxide anion instead of nitric oxide.[26]

INSULIN RESISTANCE AND CARDIOVASCULAR DISEASE

Insulin resistance is defined as " resistance to effects of insulin on glucose uptake, metabolism and storage". Insulin resistance leads to decreased uptake of glucose in muscle and adipose tissue and an inability of the hormone to suppress hepatic gluconeogenesis. The end result of all these, is the β cell failure leading to impaired glucose tolerance and diabetes mellitus.



(Reproduced from Robbins and Cotran, Textbook on Pathologic basis of disease, 7th edition, page 1195)

Thus insulin resistance leads to cardiovascular disease through hyperglycemia and related metabolic abnormalities. The occurrence of multiple metabolic abnormalities in an individual is termed as **Insulin resistance syndrome** or **syndrome X** or **metabolic syndrome**.

COMPONENTS OF METABOLIC SYNDROME[27]

VARIABLE	DEFINING LEVELS
Hyperglycemia	> 110mg/dL
Dyslipidemia	HDL Cholesterol
	Men <40mg/dL
	Women <50mg/dL
	Triglycerides >150mg/dL
Obesity	Waist circumference
	Men >40 inches
	Women >35 inches
Hypertension	Blood pressure >130/85 mm Hg
Age	Men >45years
	Women >55years

Insulin resistance independently predicts the cardiovascular risk. Howard G et al[28], have stated that insulin resistance positively correlates with atherosclerosis, as assessed by carotid intima media thickness. Lempianen P et al [29], state that severity of insulin resistance directly correlates with the incidence of myocardial infarction.

DIABETIC DYSLIPIDEMIA

The primary lipid disorders in diabetes are elevated triglycerides, decreased high density lipoprotein cholesterol and an increase in small

dense low density lipoproteins. Increased delivery of free fatty acids to the liver due to excess adipose tissue efflux and impaired skeletal muscle uptake increases hepatic production of very low density lipoprotein and cholesteryl ester synthesis. Over production of triacylglycerol rich lipoprotein and impaired clearance by lipoprotein lipase leads to hyper triglyceridemia in diabetes.[30]

Free fatty acids also produce reactive oxygen species by activating intracellular enzymatic oxidant sources like protein kinase C, NADPH oxidase and endothelial nitric oxide syntheses.[31] Triglyceride levels correlates inversely with high density lipoprotein levels as cholesteryl ester transfer protein mediates exchange of cholesterol from high density lipoprotein from very low density lipoprotein.[30] Miller M, et al[32] have noticed in their study of distribution of lipids in 8500 men with coronary artery disease, that elevated triglycerides and low high density lipoprotein is more common than elevated total and low density lipoprotein cholesterol in diabetic patients with coronary artery disease.

In addition to the changes in the concentration of high density lipoprotein, functional defects occur in diabetic subjects, leading to decreased capacity to prevent low density lipoprotein oxidation, promoting atherogenesis.[33] Increased concentrations of small, dense low density lipoprotein in diabetic persons results from abnormal cholesterol and triglyceride transfer between very low density lipoprotein and low

density lipoprotein, these molecules are proatherogenic. They bind to intimal proteoglycans, which enhances their retention in the intima, promoting the oxidative modification and are taken up by macrophages and smooth muscle cells.[30]

OTHER PATHO PHYSIOLOGICAL CHANGES LEADING TO ATHEROSCLEROSIS

Platelet abnormalities are common in diabetics, caused by activation of protein kinase C, decreased production of platelet derived nitric oxide, leading to oxidative stress.[34] Diabetes impairs platelet calcium homeostasis and results in the abnormal platelet shape, aggregation and thromboxane formation. In addition, platelets in patients with diabetes have increased expression of adhesive glycoproteins. [35]

Type 2 diabetes increases plasminogen activator inhibitor type 1 levels, impairing fibrinolytic capacity in atherosclerotic lesions.[36] Diabetes also reduces the levels of endogenous anticoagulants.[37] Diabetes increase vascular smooth muscle growth and the migration of the smooth muscle cells into the atherosclerotic lesions.

Diabetes reduces the production of collagen, and increase the production of matrix metalloproteinases, there by decreasing the

stability of fibrous cap. This can lead to increased incidence of plaque rupture and thrombosis.[38]

By all these mechanisms diabetes predisposes to accelerated process of atherosclerosis and increase the patient susceptibility to the thrombotic complications of atherosclerosis.

C – REACTIVE PROTEIN

A Sensitive marker of inflammation and tissue damage, C –reactive protein has been named for its capacity to precipitate the somatic C- polysaccharide of streptococcus pneumoniae. It is the first among the acute phase protein to be described.

Originally, C –reactive protein was measured in clinical laboratories to detect active infection and inflammation. The levels of C- reactive protein in these conditions varied from 3mg/L to 150mg/L. This wide range of C –reactive protein levels shows the lack of sensitivity in various infection and inflammation. It is not possible to apply such an assay for the determination of cardiovascular risk in apparently healthy men and women as the assay lacks sensitivity.

Immunoassays for C- reactive protein were developed with greater sensitivity. The development of these high sensitivity assays has gained importance in predicting the coronary artery disease by detecting the inflammatory changes caused by the pathogenesis of atherosclerosis. The recent method available is very sensitive for the measurement of C- reactive protein is capable of identifying a very narrow range between 0-3 mg/L.

So high sensitivity C- reactive protein measurements are very sensitive and can identify even minimal inflammatory reactions. It is

possible to use high sensitivity C- reactive protein for the identification of inflammatory changes associated with atherosclerosis.[39]

STRUCTURE OF C- REACTIVE PROTEIN

C- reactive protein belongs to the pentraxin (from the Greek word penta- five and ragos- berries) family of calcium dependent ligand binding plasma proteins. The C- reactive protein molecule has a molecular weight of 115,135 Da and it is composed of five identical non- glycosylated polypeptide subunits of molecular weight 23,027Da, each containing 206 amino acid residues.

The protomers are non- covalently associated, with cyclic pentameric symmetry. Each protomer has the characteristic "lectin fold" composed of a two layered β sheet with flattened jelly roll topology. Two calcium ions are bound 4Å apart by protein side chains and this is the site of ligand binding. C- reactive protein binds with highest affinity to phosphocholine residues, and a variety of other autologous and extrinsic ligands, and aggregates or precipitates the molecular structures bearing these ligands. When C- reactive protein is ligand bound, it is recognized by C1q and potently activates the classical complement pathway, C3 the main adhesion molecule of the complement system and the terminal membrane attack complex C5- C9. Bound C- reactive protein also provides binding sites for factor H, there by regulates

alternative pathway amplification and C5 convertases. These may lead to cell lysis.

The three dimensional structure of C reactive protein, shows the presence of deep, extended cleft in each protomer on the face of pentamer opposite to that containing phosphocholine binding site. This cleft is of functional importance. It is deep at its origin, but becomes shallow at the inner edge of the protomer , the shallow end of the pocket is bounded by the 112- 114 loop, residues 86- 92, the C- terminus of the pentraxin alpha helix 169-176, particularly tyrosine (175).This functions as a C1q binding site of C- reactive protein. Asp 112,Tyr 175 are important contact residues for C1q binding. Glu 88 influences the conformational change in C1q necessary for complement activation. Asn 158 and His 38 contribute to the geometry of the binding site.[39, 40.]

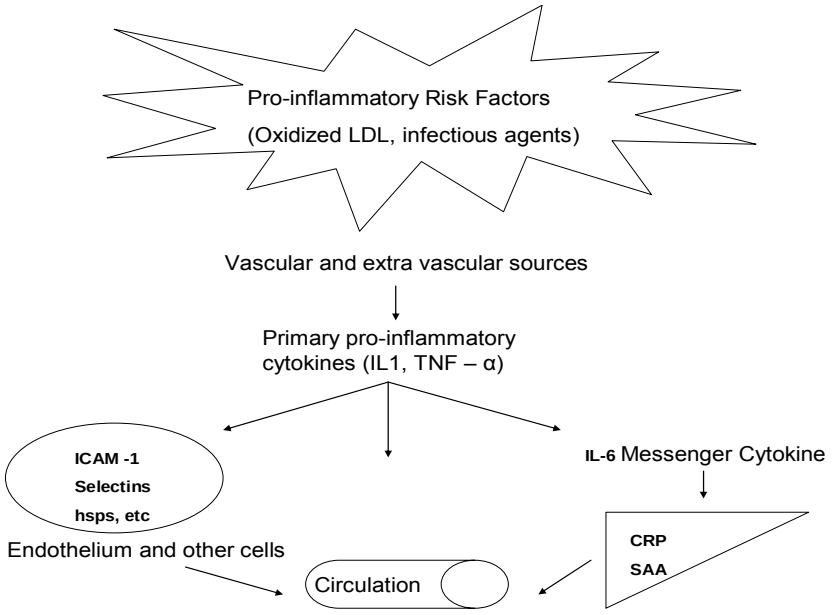
SYNTHESIS AND CIRCULATION OF C- REACTIVE PROTEIN

Atherosclerosis, and process of athero thrombotic events are inflammatory processes. This leads to expression of proinflammatory cytokines Interleukin- 1, which inturn expresses Interleukin- 6 which leads to increased expression of C-reactive protein gene, thereby the production of C- reactive protein is increased.

In healthy young adult volunteer blood donors, the median C- reactive protein concentration is 0.8 mg/L. The synthesis

of C-reactive protein starts very rapidly after the initial stimulus, serum concentrations rise above 5mg/L by about 6 hours, and peak about 48 hours. The plasma half life of C- reactive protein is 19 hours, and it is constant under all conditions. Hence the sole determinant of C- reactive protein concentration is the synthesis rate in the liver, which reflects directly the intensity of the pathological process. The circulating value of C- reactive protein, reflects the ongoing inflammation more accurately than other acute phase reactants.

The C- reactive protein values show no diurnal variation and are unaffected by diet. Liver failure impairs C- reactive protein production. Its concentration is also independent of age, smoking, diabetes, hypertension and dyslipidemia. Use of oral contraceptives and post menopausal systemic hormone replacement therapy are associated with high C- reactive protein levels.[41, 42]



(Reproduced from James T. Willerson, Paul M. Ridker: Inflammation as a Cardiovascular risk factor and Vascular effect of Statins. **Circulation** 2004, 109: II2 –II10)

C- REACTIVE PROTEIN AND PATHOGENESIS OF ATHEROSCLEROSIS

Numerous studies, have provided a positive correlation between elevated high sensitivity C-reactive protein levels and the underlying atherosclerotic disease process. The elevated levels of C- reactive protein, also predicts the future athero thrombotic events, including coronary events, stroke and progression of peripheral arterial disease. The predictive power for future cardiovascular disease, is stronger for

C- reactive protein than for Low density lipoprotein cholesterol. Physicians health study and Women health study showed that the predictive value of high sensitivity C- reactive protein was higher than the traditional biochemical risk marker for coronary heart disease like total cholesterol, high density lipoprotein and low density lipoprotein or novel markers like lipoprotein a, homocysteine, apolipoprotein A and apolipoprotein B.[43]

The reference values for low, moderate, high cardiovascular risk group has been set by AHA/CDC panel and are less than 1mg/L, 1mg/L to 3mg/L, greater than 3mg/L respectively. It has also recommended that high sensitivity C- reactive protein should be included in the global risk assessment of coronary heart disease in the primary prevention setting.[44]

Recent studies have proposed that C- reactive protein is not only a marker of inflammation in atherosclerosis, but an actual participant in the disease process. It is shown that C-reactive protein enhances expression of local endothelial cell surface adhesion molecules, monocytes chemotactic protein-1, endothelin-1, plasminogen activator inhibitor-1, reduce endothelial nitric oxide bioactivity and increase the tissue uptake of modified low density lipoprotein by binding to it. In addition, C- reactive protein once formed may activate complement, sustain inflammation and induce monocytes to produce Interleukin 6

and interleukin 7, that in turn induces C-reactive protein production. Thus C- reactive protein once formed can activate its own synthesis.[45]

With all these evidences, the present study is aimed at measuring C- reactive protein with the high sensitivity assays and correlates the values with traditional biochemical risk markers of atherosclerosis like total cholesterol, high density lipoprotein and low density lipoprotein.

MATERIALS AND METHODS

The study was conducted after getting the approval from the ethical committee of Stanley medical college. Hundred subjects were chosen for the study. All of them were males of age group 45 years- 70 years and an informed consent was obtained from all of them.

Fifty subjects, who were diagnosed to be diabetic, as per criteria of American diabetes association and WHO 2004, with no evidence of atherosclerotic coronary artery disease in Electrocardiogram formed the control group. They were selected from the department of Diabetology, Stanley Medical College.

Fifty subjects, who were diagnosed to be diabetic, as per the criteria of American diabetes association and WHO 2004 and associated with evidence of atherosclerotic coronary artery disease in Electrocardiogram formed the case group and they were selected from the department of Cardiology, Stanley medical college.

INCLUSION CRITERIA

1) Subjects diagnosed to be diabetic with any one of the following criteria in two occasions

Symptoms of diabetes plus random plasma glucose concentration ≥200 mg/dL. (or)

Fasting (defined as no caloric intake for at least 8 hours) plasma glucose ≥ 126 mg/dL. (or)

Two hours plasma glucose ≥200 mg/dL during an oral glucose tolerance test.

2) Presence of atherosclerotic coronary artery disease evidenced by Electrocardiogram changes like

Abnormal ST-T wave changes (ST segment elevation or depression or deep symmetrical T wave inversion) and formation of new Q waves.

EXCLUSION CRITERIA

1) Presence of hypertension, defined by resting systolic blood pressure > 140 mmHG and diastolic blood pressure > 90 mm HG.

2) Presence of Obesity, defined by body mass index more than 30 kg/m^2.

3) Presence of malignancy and concomitant systemic diseases like rheumatic disease, chronic liver disease, renal disorder and sepsis.

4) Critically ill patients, smokers and alcoholics.

5) Ongoing infectious diseases.

6) Having known thrombotic or bleeding disorders.

7) Persons on Statin therapy.

BLOOD COLLECTION

5mL of Blood samples were collected by vene puncture with strict aseptic precaution after an overnight fast for at least 12 -14 hours. 1mL of the sample was aliquoted into a test tube containing EDTA and Sodium fluoride mixture (0.5mg+ 1mg). The sample was centrifuged and plasma separated and the analysis for glucose was done immediately.

Remaining 4 mL of the sample was allowed to clot and the serum was separated after centrifugation and the analysis of total cholesterol, triglycerides, high density lipoprotein was done immediately and 0.5 mL of serum was stored at -20˚C for analysis of high sensitivity C- reactive protein.

ESTIMATION OF PLASMA GLUCOSE

The tests are performed in the reagent kit by **GLUCOSE OXIDASE-PEROXIDASE METHOD**.

Principle

Glucose is oxidized by Glucose Oxidase (GOD) into gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of Peroxidase (POD) oxidizes chromogen 4-amino antipyrine and phenolic compound to form a pink coloured compound.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{phenolic Compound} + \text{4-amino antipyrine} \xrightarrow{\text{Peroxidase}} \text{Pink Compound} + H_2O$$

The intensity of the colour formed is proportional to the glucose concentration and is measured at 505nm.

Reagents

Buffer/ Enzymes/ Chromogen

Phosphate buffer	95 m mol/L
4- amino antipyrine	0.2 m mol/L
p- Hydroxy benzoic acid	5.9 m mol/ L

Glucose Oxidase	≥ 5000 U/L
Peroxidase	≥ 5000 U/L

Standard

Glucose	100mg/dL

General system parameters

Reaction type	End point
Reaction Slope	Increasing
Wavelength	505 nm
Flow cell temperature	30˚C
Incubation	30 minutes /15 minutes RT/ 30˚C
Sample volume	10µL
Reagent volume	1000 µL
Zero setting with	Reagent blank
Standard	100 mg/dL

Procedure

	Blank	Standard	Test
Reagent	1mL	1mL	1mL
Standard	--	10 µL	--
Sample	--	--	10 µL

Mix well and incubate for 15 minutes.

Calculation

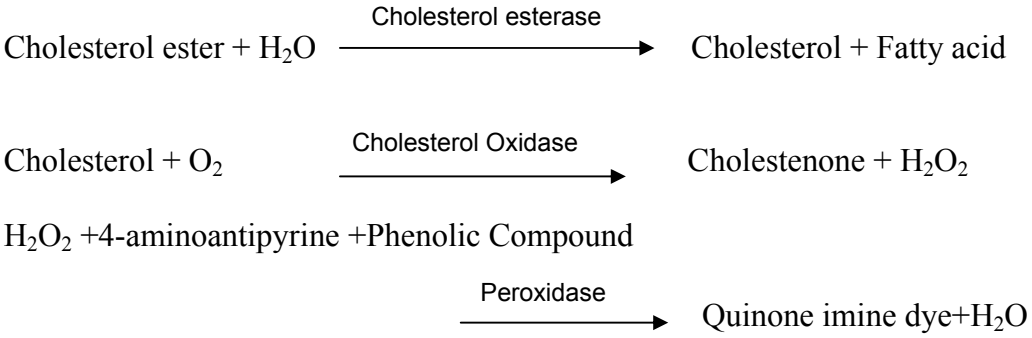
$$\text{Glucose (mg/dL)} = \frac{\text{Abs. of sample}}{\text{Abs. of Standard}} \times \text{Concentration of the standard}$$

ESTIMATION OF TOTAL CHOLESTEROL

The tests are performed in the reagent kit by **ENZYMATIC CHOLESTEROL ESTERASE METHOD**.

Principle

The free cholesterol liberated from the cholesterol esters by cholesterol esterase is oxidized by cholesterol oxidase to cholestenone with the simultaneous production of hydrogen peroxide. The hydrogen peroxide reacts with 4- amino antipyrine and a phenolic compound in the presence of peroxidase to yield a red coloured complex.



The concentration of cholesterol in the sample is directly proportional to the intensity of the red coloured complex which is measured at 500 nm.

Reagents

Reagent 1 (Enzymes/ Chromogen)

Cholesterol esterase	≥ 200 U/L
Cholesterol oxidase	≥ 250 U/L
Peroxidase	≥ 1000U/L
4- Amino antipyrine	0.5 m mol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.90	50 m mol/L
Phenol	24 m mol/L

Sodium cholate 0.5 m mol/L

Standard:

Cholesterol 200 mg/dL

Reconstituted Reagent

Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1A.

General system parameters

Reaction type	End point
Reaction Slope	Increasing
Wavelength	500 nm
Flow cell temperature	30
Incubation	5 minutes at 37˚C
Sample volume	10µL
Reagent volume	1000 µL
Zero setting with	Reagent blank
Standard	200 mg/dL

Procedure

	Blank	Standard	Test
Reagent	1mL	1mL	1mL
Standard	--	10 µL	--
Sample	--	--	10 µL

Incubate for 5 minutes at 37˚C.

Calculation

$$\text{Total Cholesterol (mg/dL)} = \frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Concentration of Std.}$$

ESTIMATION OF TRIACYLGLYCEROL

The tests are performed in the reagent kit by **Enzymatic Colorimetric method**.

Principle

Lipoprotein lipase catalyzed hydrolysis of triglycerides yield glycerol which is phosphorylated by Glycerol kinase using ATP to glycerophosphate which upon oxidation yields dihydroxy acetone phosphate and hydrogen peroxide. The hydrogen peroxide is used to

oxidize 4 amino antipyrine and N- Ethyl-N- Sulfopropyl-N-anisidine (ADPS) and water.

$$\text{Triacylglycerol} + H_20 \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{Glycerol 1Phosphate} + \text{ADP}$$

$$\text{Glycerol 1-Phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate Oxidase}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{DHBS} \xrightarrow{\text{Peroxidase}} \text{Quinoneiminedye} + H_20$$

DAP –Dihydroxy acetone phosphate

DHBS – 3,5 Dichloro 2-hydroxy benzene sulfonate

The intensity of the purple coloured complex formed during the reaction is directly proportional to the triacylglycerol concentration in the sample and it is measured at 546nm.

Reagents

Reagent 1 (Enzymes / Chromogen)

Lipoprotein Lipase	≥ 1100 U/L
Glycerol Kinase	≥ 800 U/L
Glycerol -3 – phosphate oxidase	≥ 5000 U/L

Peroxidase	≥ 350 U/L
4- Amino antipyrine	0.7 m mol/L
ATP	0.3 m mol/L

Reagent 1A (Buffer)

Pipes buffer, pH 7.50	50 m mol/L
ADPS	1 m mol/L
Magnesium salt	15 m mol/L

Standard

Triacylglycerol	200 mg/dL.

Reconstituted Reagent

Dissolve the contents of one bottle of reagent 1 with one bottle of reagent 1A.

General system parameters

Reaction type	End point
Reaction Slope	Increasing
Wavelength	505 nm
Flow cell temperature	30˚C
Incubation	5 minutes at 37˚C
Sample volume	10µL
Reagent volume	1000 µL
Zero setting with	Reagent blank
Standard	200 mg/dL

Procedure

	Blank	Standard	Test
Reagent	1mL	1mL	1mL
Standard	--	10 µL	--
Sample	--	--	10 µL

Mix well and incubate for 5 minutes at 37˚C.

Calculation

$$\text{Triacylglycerol (mg/dL)} = \frac{\text{Abs. of sample}}{\text{Abs. of Standard}} \times \text{Concentration of the Standard}$$

ESTIMATION OF HIGH DENSITY LIPOPROTEIN:

The tests are performed in the reagent kit by **PHOSPHOTUNGSTATE METHOD**.

Principle

Chylomicrons, very low density lipoprotein, low density lipoprotein fractions in serum or plasma are separated from high density lipoprotein (HDL) by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using Cholesterol esterase, Peroxidase method.

Reagents

Reagent 1 (Enzymes/ Chromogen)

Cholesterol esterase	≥ 200 U/L
Cholesterol oxidase	≥ 250 U/L
Peroxidase	≥ 1000U/L

4- Amino antipyrine 0.5 m mol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.90 50 m mol/L

Phenol 24 m mol/L

Sodium cholate 0.5 m mol/L

Reagent 2 (Precipitating Reagent)

Phosphotungstic acid 2.4 m mol/L

Magnesium Chloride 39 m mol/L

Standard

HDL Cholesterol 50 mg/dL

Procedure

1. Precipitation

   dispense into centrifuge tubes.

	Test
Sample	200 µL
Precipitating reagent	200 µL

Mix well. Centrifuge at 3500 – 4500 rpm for 10 minutes. Separate the clear supernatant immediately and determine the cholesterol content.

General system parameters

Reaction type	End point
Reaction Slope	Increasing
Wavelength	500 nm
Flow cell temperature	30˚
Incubation	5 minutes at 37˚C
Sample volume(Supernatant)	20µL
Reagent volume	1000 µL
Zero setting with	Reagent blank
Standard	100 mg/dL

Procedure

	Blank	Standard	Test
Reagent	1mL	1mL	1mL
Standard	--	20 µL	--
Sample	--	--	20 µL

Mix well and incubate for 5 minutes at 37˚C.

Calculation

$$\text{HDL Cholesterol (mg/dL)} = \frac{\text{Abs. of sample}}{\text{Abs. of Standard}} \times \text{Concentration of standard}$$

CALCULATED PARAMETERS:

Friedwald's Formula:

Very Low Density Lipoprotein: $\dfrac{\text{Triacylglycerol}}{5}$

Low Density Lipoprotein: Total Cholesterol – HDL – $\dfrac{\text{Triacylglycerol}}{5}$

ESTIMATION OF HIGH SENSITIVITY C -REACTIVE PROTEIN

Particle enhanced Immunoturbidimetric test

Principle

The CRP – ultra sensitive assay is a quantitative turbidimetric test for the measurement of low levels of C- reactive protein in human serum or plasma. Latex particles coated with specific anti- human CRP are agglutinated when mixed with samples containing CRP. The agglutination causes an absorbance change, dependent upon the CRP contents of the patient sample that can be quantified by comparison from calibrator of known CRP concentration.

Reagents Reagent 1 (Diluent)

Tris buffer 20 m mol/L

Sodium azide 0.95 g/L

pH 8.2

Reagent 2 (Latex reagent)

Latex particles coated with goat IgG anti- human CRP

Sample : Clear serum sample

Procedure

Dispense	**Calibrator**	**Sample**
Working reagent	1000 µL	1000 µL
Calibrator	10 µL	--
Sample	--	10 µL

Measure the absorbance at 540 nm after 10 seconds and after 4 minutes of sample addition. The absorbance differences are calculated for each CRP calibrator and the values are plotted against the CRP concentration in a calibration curve. CRP Concentration in the sample is calculated by interpolation of the of the absorbance values in the calibration curve.

Reference values : 0 - 3 mg/L.

Absorbance of hs-CRP calibrators:

Concentration of Standards(mg/L)	Absorbance
0.000	0.000
0.585	0.015
1.170	0.030
2.925	0.080
5.850	0.129

RESULTS AND STATISTICAL ANALYSIS

A total of 100 patients were included in the present study. Out of the 100, 50 were study group (Diabetes mellitus with Atherosclerotic coronary artery disease) and other 50 were controls (Diabetes mellitus without Atherosclerotic coronary artery disease).

AGE DISTRIBUTION AMONG THE STUDY AND CONTROL GROUP

Male patients in the age group 45 years- 70 years were taken for the study. Both the Study and control group patients were age matched. The mean age of the study group is 54.94 and the mean age of the control group is 52.88.

Table 1

Group	N	Mean	Standard Deviation	Student independent t test
Control	50	52.88	6.08	t=1.67
Study	50	54.94	6.28	p=0.09
				Not Significant

Qualitative variable (age) is given as frequency with their percentages.

Table 2

	Control		Study		Chi Square test
	N	**%**	**N**	**%**	
Age <50	19	61.3	12	38.7	p=0.31
51-60	25	45.5	30	54.5	Not Significant
>60	6	42.9	8	57.1	
Total	50	50.0	50	50.0	

The Serum levels of total cholesterol, triacylglycerol, high density lipoprotein, hs- CRP were estimated for all the patients taken for the study. Low density lipoprotein and very low density lipoprotein values were calculated. The values obtained in both the study and control group are presented in the Master chart I and II.

Mean and Standard deviation were calculated for the Quantitative variables (total cholesterol, triacylglycerol, high density lipoprotein, low density lipoprotein and very low density lipoprotein, hs- CRP) in both study and control group. The values were analyzed using Student's Independent `t´ test. The results are presented in tables 3-6.

Correlation between hs –CRP and other variables were analyzed using Pearson's correlation analysis. The results are presented in tables 7& 8.

The Receiver Operating Characteristic (ROC) Curves are plotted for low density lipoprotein, LDL/HDL ratio, total Cholesterol/HDL ratio and hs- CRP and it is presented in figures 1-4.

COMPARISON OF THE BIOCHEMICAL PARAMETERS IN THE STUDY AND CONTROL GROUP:

Table 3

Parameter	Group	Mean	Standard Deviation	Student Independent t test
Plasma Glucose	Control Study	120.66 125.50	37.98 33.90	t=0.67 p=0.50 Not Significant
Total Cholesterol	Control Study	178.84 190.42	42.91 38.16	t=1.42 p=0.16 Not Significant
Triacylglycerol	Control Study	128.90 158.76	66.39 17.16	t=2.08 p=0.04 Significant
High density lipoprotein	Control Study	38.08 33.30	9.27 8.12	t=2.74 p=0.01 Significant
Low density lipoprotein	Control Study	107.54 131.34	38.55 34.39	t=3.25 p=0.05 Significant
Very Low density lipoprotein	Control Study	25.68 29.78	13.28 3.41	t=2.01 p=0.05 Significant
Total Cholesterol/ High density lipoprotein	Control Study	4.94 5.91	1.43 1.34	t=3.49 p=0.001 Significant
Low density Lipoprotein / High density lipoprotein	Control Study	2.99 4.04	1.21 1.14	t=4.43 p=0.001 Significant
High Sensitivity C-reactive protein	Control Study	1.74 6.51	0.78 1.02	t=26.15 p=0.001 Significant

COMPARISON OF THE BIOCHEMICAL PARAMETERS IN AGE MATCHED STUDY AND CONTROL GROUP

Table 4

Age <50 years

Parameter	Group	Mean	Standard Deviation	Student Independent t test
Plasma Glucose	Control Study	126.79 126.92	35.28 25.09	t=0.01 p=0.99 Not Significant
Total Cholesterol	Control Study	176.37 190.42	41.57 32.25	t=0.99 p=0.33 Not Significant
Triacylglycerol	Control Study	127.74 134.75	50.39 22.02	t=0.45 p=0.65 Not Significant
High density lipoprotein	Control Study	40.95 35.83	10.01 8.11	t=1.48 p=0.15 Not Significant
Low density lipoprotein	Control Study	104.58 129.09	45.38 30.61	t=1.64 p=0.11 Not Significant
Very Low density lipoprotein	Control Study	25.95 26.50	9.99 4.40	t=0.47 p=0.64 Not Significant
Total Cholesterol/ High density lipoprotein	Control Study	4.63 5.51	1.62 1.44	t=1.52 p=0.14 Not Significant
Low density Lipoprotein / High density lipoprotein	Control Study	2.81 3.55	1.48 0.99	t=1.51 p=0.14 Not Significant
High Sensitivity C-reactive protein	Control Study	1.55 7.05	0.61 1.01	t=18.96 p=0.001 Significant

COMPARISON OF THE BIOCHEMICAL PARAMETERS IN AGE MATCHED STUDY AND CONTROL GROUP

Table 5

Age 51-60 years

Parameter	Group	Mean	Standard Deviation	Student Independent t test
Plasma Glucose	Control Study	116.80 124.07	43.17 39.32	t=0.65 p=0.52 Not Significant
Total Cholesterol	Control Study	179.84 183.17	46.82 35.70	t=0.29 p=0.77 Not Significant
Triacylglycerol	Control Study	125.72 163.43	79.98 13.11	t=0.45 p=0.65 Not Significant
High density lipoprotein	Control Study	35.76 31.70	8.76 7.01	t=2.58 p=0.01 Not Significant
Low density lipoprotein	Control Study	107.40 126.30	34.84 33.27	t=2.05 p=0.04 Significant
Very Low density lipoprotein	Control Study	25.56 32.13	16.07 2.59	t=2.49 p=0.02 Significant
Total Cholesterol/ High density lipoprotein	Control Study	5.18 5.94	1.34 1.26	t=2.16 p=0.04 Significant
Low density Lipoprotein/ High density lipoprotein	Control Study	3.11 4.10	1.08 1.17	t=3.23 p=0.002 Significant
High Sensitivity C-reactive protein	Control Study	1.86 6.34	0.91 1.02	t=17.03 p=0.001 Significant

COMPARISON OF THE BIOCHEMICAL PARAMETERS IN AGE MATCHED STUDY AND CONTROL GROUP

Table 6

Age >60 years

Parameter	Group	Mean	Standard Deviation	Student Independent t test
Plasma Glucose	Control Study	117.33 128.75	21.92 25.02	t=0.88 p=0.39 Not Significant
Total Cholesterol	Control Study	182.50 217.63	35.42 46.97	t=1.53 p=0.15 Not Significant
Triacylglycerol	Control Study	132.00 142.75	33.86 17.76	t=0.77 p=0.45 Not Significant
High density lipoprotein	Control Study	38.67 35.50	7.36 11.27	t=0.59 p=0.56 Not Significant
Low density lipoprotein	Control Study	117.50 153.50	34.16 39.28	t=1.79 p=0.01 Significant
Very Low density lipoprotein	Control Study	26.63 28.63	6.80 3.50	t=0.82 p=0.42 Not Significant
Total Cholesterol/ High density lipoprotein	Control Study	4.79 6.44	1.00 1.44	t=2.38 p=0.03 Significant
Low density Lipoprotein/ High density lipoprotein	Control Study	3.06 4.53	0.80 1.11	t=2.71 p=0.02 Significant
High Sensitivity C-reactive protein	Control Study	1.90 6.35	0.66 0.87	t=10.37 p=0.001 Significant

PEARSON'S CORRELATION ANALYSIS:

Table 7

	Age	TGL	CHOL(T)	HDL	VLDL	LDL	hs CRP
LDL/HDL ratio Vs							
t value	0.16	0.65	0.40	0.48	0.07	0.55	0.28
p value	0.25	0.06	0.004	0.001	0.58	0.002	0.043
Significance	ns	ns	S	S	ns	S	S

Table 8

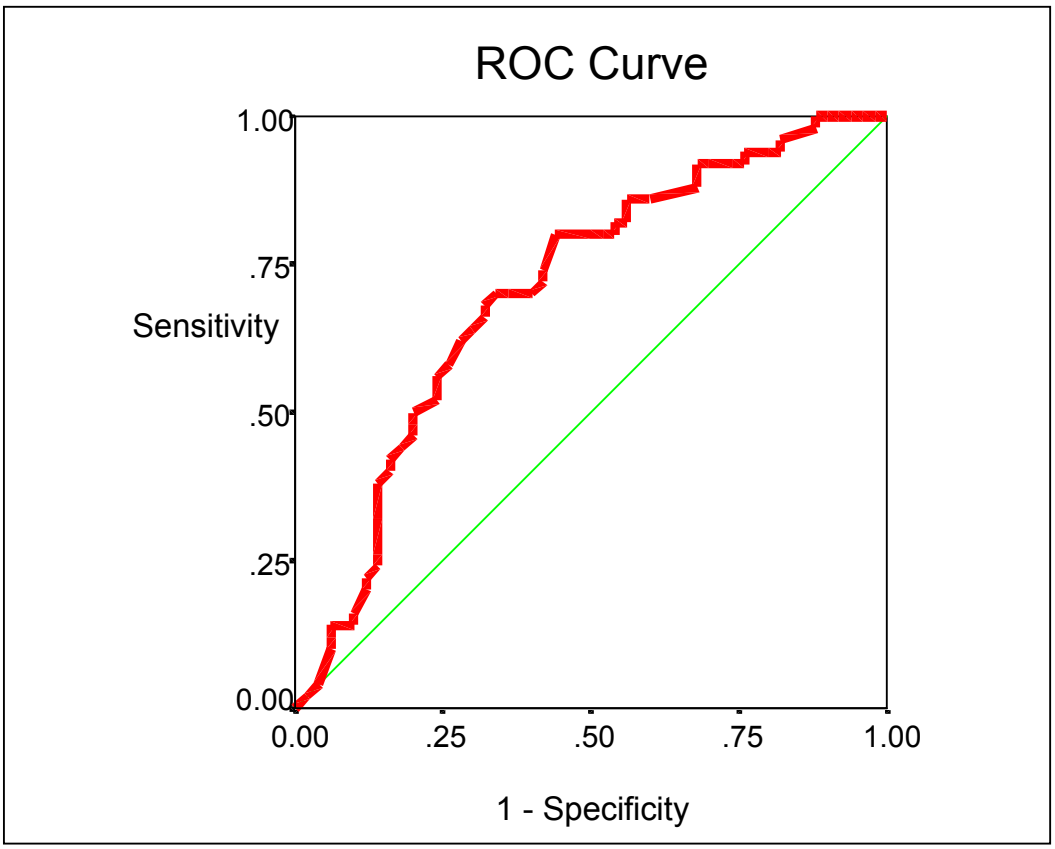
	Age	TGL	CHOL(T)	HDL	VLDL	LDL	LDL/HDL
hs CRP Vs							
t value	0.12	0.01	0.07	0.12	0.04	0.11	0.28
p value	0.37	0.90	0.59	0.40	0.78	0.44	0.043
Significance	ns	ns	ns	ns	ns	ns	S

ns- Not Significant

S - Significant

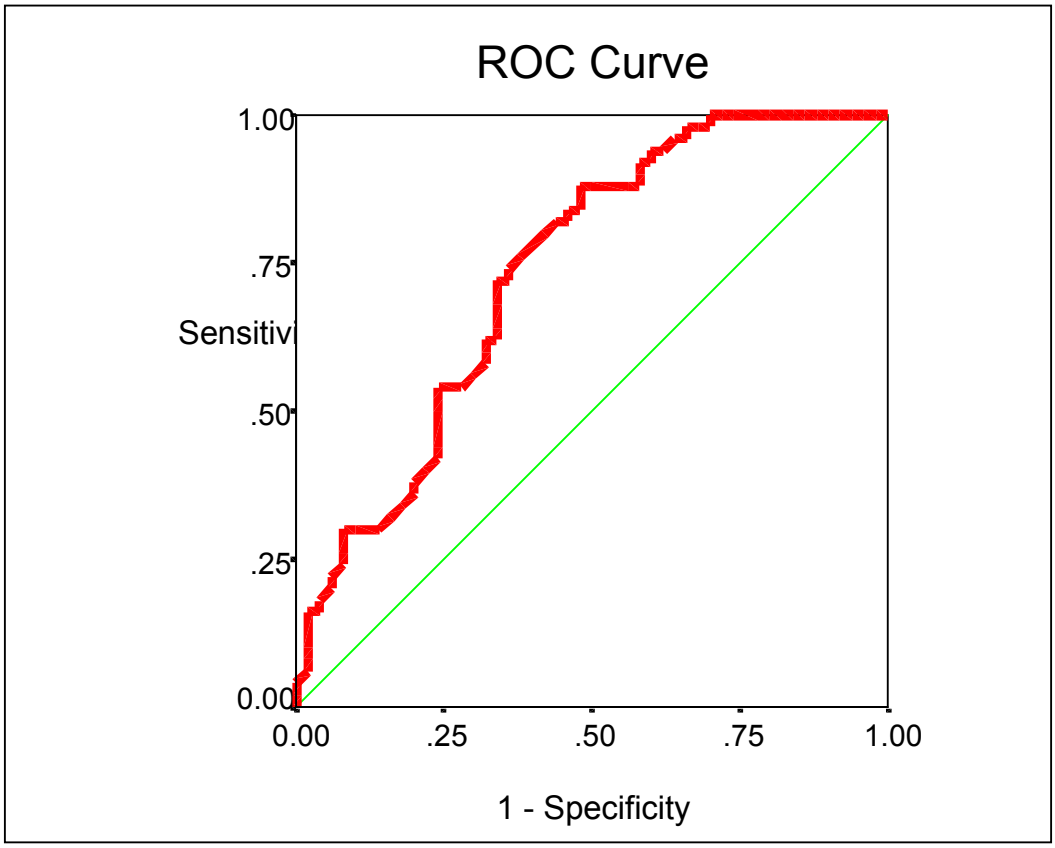
RECEIVER OPERATING CHARACTERISTIC CURVE FOR LDL

Figure 1



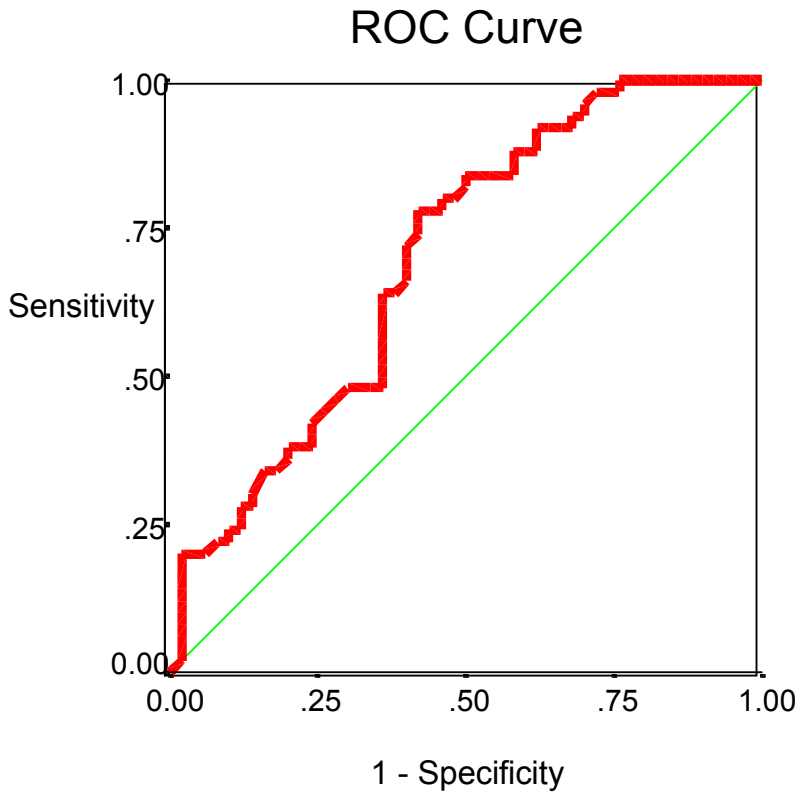
RECEIVER OPERATING CHARACTERISTIC CURVE FOR LDL/HDL ratio

Figure 2



RECEIVER OPERATING CHARACTERISTIC CURVE FOR TOTAL CHOLESTEROL/ HDL ratio

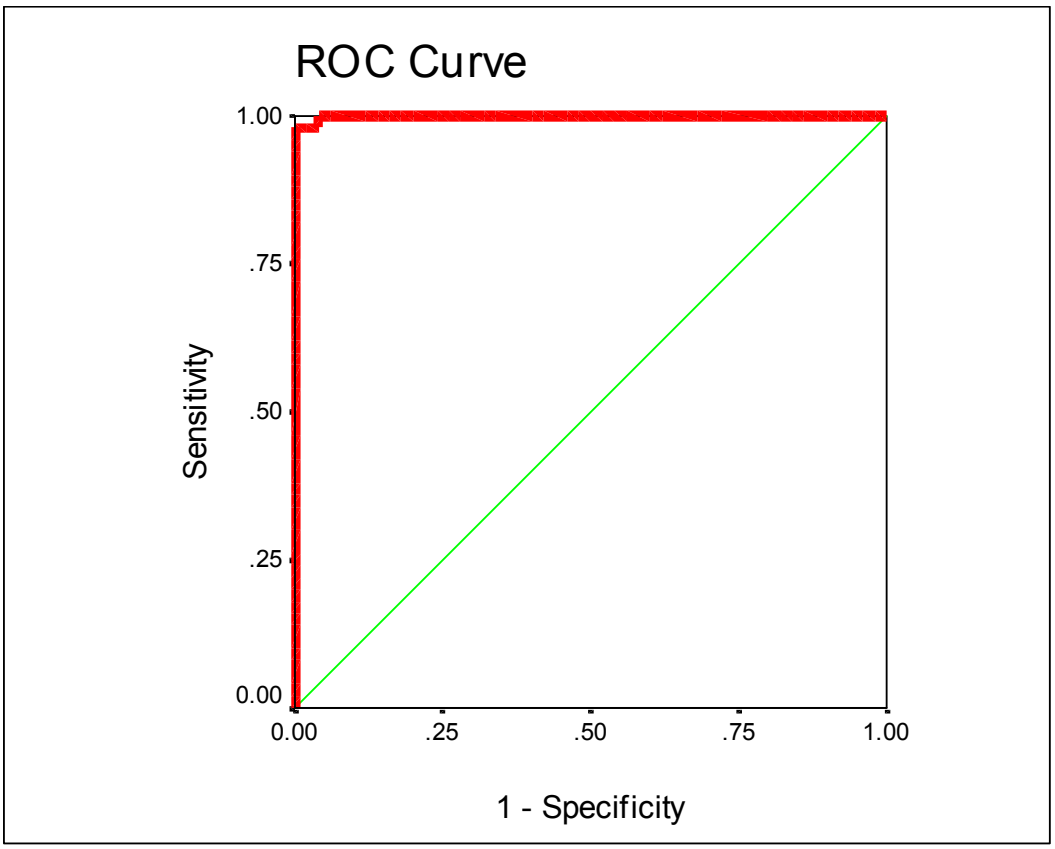
Figure 3



RECEIVER OPERATING CHARACTERISTIC CURVE

FOR hs CRP

Figure 4



DISCUSSION

Epidemiological and Clinical studies have shown strong relationship between markers of inflammation and the risk of future cardiovascular events. Inflammation can be detected locally by temperature and pH changes. It can be detected systemically by measurement of inflammatory markers.

The markers of inflammation reported so far in atherosclerosis include high sensitivity C reactive protein, interleukin-6, serum amyloid A, tumour necrosis factor- alpha, soluble intercellular adhesion molecule-1, macrophage inhibitory cytokine-1, p- Selectin, CD40 ligand.[46, 47]

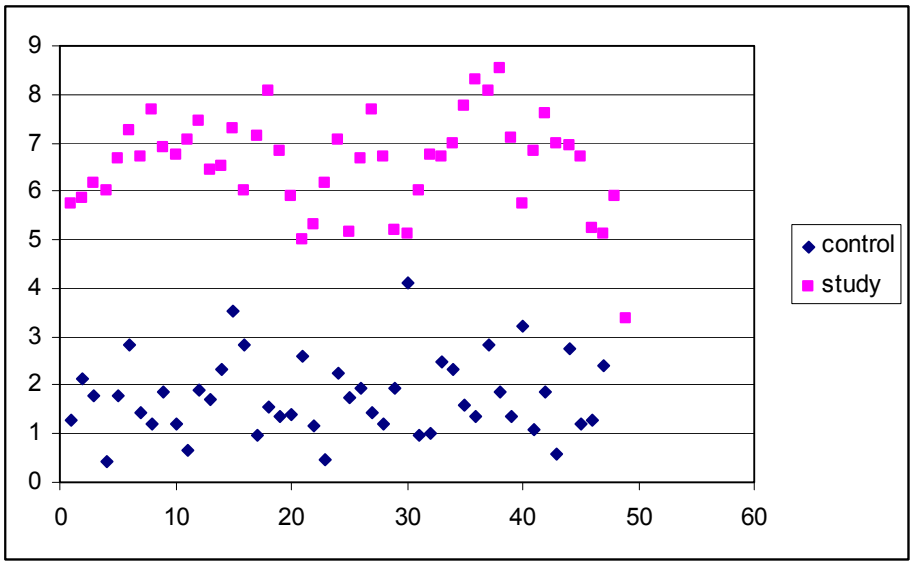
Among these various markers, high sensitivity C- reactive protein is widely studied experimentally and is easy to estimate in the laboratory. It has been shown in the Honolulu heart study, that the measurement of high sensitivity C- reactive protein can be used to identify the risk of coronary artery disease at a very early stage.[48]

The traditional risk factors used in the prediction of atherosclerosis are low density lipoprotein cholesterol, triacylglycerol, total Cholesterol, LDL/HDL ratio, while high density lipoprotein is a marker of anti atherogenic potential in an individual.

But the efficacy of these traditional risk factors is questionable in their ability to identify all the individuals at an increased risk. This has been shown in a 2003 study of more than 120,000 patients that approximately 20 percent of all coronary events occurred in the absence of any major risk factors like hyperlipidemia, hypertension, diabetes and smoking.[49]

In the present study, the mean value of hs- CRP showed a significant increase in the study group compared to the control group. The results of the present study shows a rise up to 2 fold in the levels of hs- CRP, which is consistent with the data given by Paul M. Ridker and James willerson, who documented a 1.5 to 7 fold increase of hs- CRP in patients with symptomatic atherosclerosis.[14]

SCATTER DIAGRAM OF hs- CRP LEVELS IN STUDY AND CONTROL GROUP



The control group data obtained in the present study showed that 35 out of 50 patients have hs- CRP levels in the range of 1-3 mg/L which is included in the moderate cardiovascular risk group by AHA/ CDC panel.[44]

This data coincides with the results of Li Pu, Ru Zhang et al. They have reported that hs- CRP is an independent biomarker for predicting coronary artery disease in diabetic population. In their follow up study the incidence of fatal cardiovascular events were much higher in patients who had hs- CRP in the levels of moderate to high cardiovascular risk group.[50]

Hence the patients taken as control group for the present study on the basis of normal ECG findings have to be evaluated by other tests to assess the cardiac function and must be followed up.

As both the groups in the present study are diabetics, the mean differences in the values of fasting plasma glucose concentration between the control and study groups are not significant. The values also reveal that the present study group subjects are well controlled diabetics.

The mean value of low density lipoprotein cholesterol in the study group is 131.32. The LDL goal for the patients with multiple risk factors (i.e) age> 45 years, male gender in the present study, according to National Cholesterol Education Program (NCEP) expert committee is less than 130, to reduce the risk of a major coronary event.

The study group has low density lipoprotein levels only slightly above the cutoff range. Rather than changes in the concentration of the low density lipoprotein, functional defects of low density lipoprotein, namely formation of small, dense low density lipoprotein would have occurred in the study group. These molecules are pro atherogenic. They could have predisposed the study group population to their present disease condition.

The Adult Treatment Panel III (ATPIII) classifies less than 40mg/dL HDL Cholesterol as low and more than 60mg/dL HDL

Cholesterol as high. The mean value of HDL- C in the present study group is 33.30, which is classified as low HDL- C. The LDL/HDL ratio, which is an indicator of atherogenic potential, is significantly raised in the study group, and it is due to a decrease in the HDL levels, which could have predisposed the study group population to the coronary artery disease. More over functional defects in the HDL-C is more common in diabetics leading to decreased capacity to prevent the oxidation of LDL Cholesterol, promoting atherosclerosis.[27]

The mean value of Total Cholesterol/ HDL-C ratio in the study group is 5.91. Paul M. Ridker in his study has reported Total Cholesterol/ HDL-C ratio of more than 5.5 in men and 5.9 in women, strongly correlate with incident cardiovascular disease. Since the value of Total Cholesterol/ HDL-C ratio is one of the strongest predictors of cardiovascular risk, the high value of Total Cholesterol/ HDL-C ratio in the present study is consistent with the previous studies.[47]

As all the patients taken for the study were diabetics, the mean difference between the values of total Cholesterol in the control and study groups did not show any statistical significance. The mean value of triacylglycerol in the study group is 158.90, which is more than the cutoff proposed by ATP III as 150 mg/dL. Hence the changes in the lipoprotein concentration with its functional abnormalities due to diabetes could have predisposed to coronary artery disease. It was found

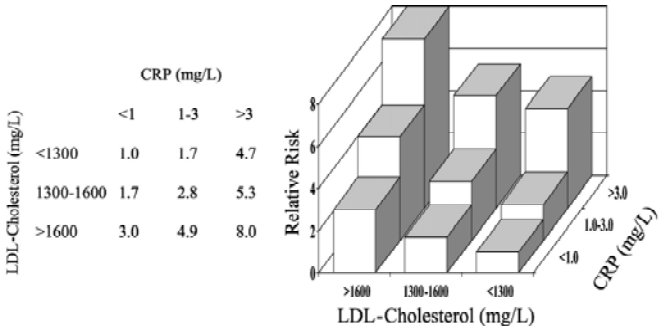
that most of the patients taken for the study were aware of the dietary control of the lipids and exercise for their ailment. This is probably one reason for the lipid levels in the study group being very close to the control.

The difference in the age matched study group did not show a uniform significance in any of the measured parameters except hs-CRP. This is probably because of the small number of patients taken for the study.

The Receiver Operating Characteristic Curves of the lipid parameters low density lipoprotein, LDL/HDL ratio, Total Cholesterol/ HDL ratio and hs-CRP reveal that the hs-CRP assay curve is above the assay curves of the lipid profile at all the points. Hence hs-CRP is a better assay for predicting the Cardio vascular risk.

In the Pearson's Correlation analysis, there is a significant correlation noticed between hs- CRP and LDL/HDL ratio. Since there is no significant linear correlation between hs- CRP and other lipid parameters, the risk of cardiovascular disease can be calculated by the algorithm proposed by Rifai N and Ridker employing the cutoff values of LDL and hs-CRP as proposed by NCEP panel.[51]

Proposed algorithm for risk assessment of coronary heart disease in men and women



The prognostic additive effect of hs- CRP to the lipid screen is very useful in the diabetic population, because the relative risk in these patients can be calculated and the patients on moderate to high risk group can be diagnosed earlier, because most diabetic individuals do not exhibit classical anginal symptoms.

CONCLUSION

High Sensitivity C- reactive protein is useful as a marker for atherosclerotic Coronary artery disease in Diabetes mellitus. Any patient with increased High Sensitivity C- reactive protein levels must be investigated for atherosclerotic Coronary artery disease.

SCOPE FOR FUTURE STUDY

Tissue necrosis is a potent acute phase stimulus, and, following myocardial injury, there is a major CRP response and the magnitude of this response, reflects the extent of myocardial necrosis. C-reactive protein can also be used to predict the outcome after myocardial infarction.

CRP is co -deposited with activated complement within all myocardial infarct and CRP response reflects not only the tissue damage, but may also contribute to the severity of ischaemic myocardial injury.

If these observations are confirmed, measurement of CRP may become an indication for prophylactic anti atherosclerotic therapy in apparently low risk individuals.

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MASTER CHART FOR CONTROL GROUP

S.No	Age	Group	Glucose (mg/dL)	Total Chol (mg/dL)	Trig (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	TC/HDL Ratio	LDL/HDL Ratio	Hs-CRP (mg/L)
1	47	1	110	145	72	59	72	14	2.45	1.22	0.78
2	45	1	96	161	255	46	64	51	3.5	1.39	1.28
3	50	1	126	190	256	46	93	51	4.13	2.02	2.15
4	54	1	85	156	270	42	59	54	3.71	1.40	1.77
5	45	1	139	150	86	35	46	18	4.28	1.31	0.43
6	45	1	116	148	126	51	50	25	2.90	0.98	1.77
7	60	1	153	181	283	33	92	56	5.48	2.78	2.82
8	45	1	98	154	155	49	74	31	3.14	1.51	1.45
9	60	1	86	171	165	45	93	33	3.80	2.06	1.21
10	45	1	112	167	141	47	92	28	3.55	1.95	1.87
11	54	1	180	200	128	58	120	22	3.44	2.06	1.21
12	48	1	162	175	100	42	113	20	4.16	2.69	0.66
13	51	1	77	142	132	32	83	26	4.43	2.59	1.91
14	49	1	92	173	145	30	114	29	5.76	3.80	1.70
15	46	1	176	181	127	30	125	25	6.03	4.16	2.33
16	55	1	202	284	364	38	173	73	7.47	4.55	3.52
17	65	1	138	151	200	25	86	40	6.04	3.44	2.82
18	46	1	96	111	78	37	58	16	3.00	2.14	0.98
19	53	1	85	190	111	33	135	22	5.75	4.09	1.56
20	58	1	140	192	115	36	133	23	5.33	3.69	1.35
21	51	1	120	171	122	40	107	24	4.27	2.67	1.38
22	49	1	110	190	105	30	139	21	6.33	4.63	2.61
23	47	1	106	188	98	53	115	20	3.54	2.16	1.17
24	51	1	102	159	65	38	108	13	4.18	2.84	0.47
25	54	1	88	176	115	52	101	23	3.38	1.94	2.26
26	63	1	96	194	114	42	130	23	4.61	3.09	1.73
27	66	1	110	216	124	47	144	25	4.59	3.06	1.94
28	70	1	106	138	116	40	75	23	3.45	1.87	1.45
29	54	1	116	106	151	22	53	30	4.81	2.40	1.21
30	51	1	98	135	156	32	72	31	4.21	2.25	1.94
31	58	1	256	158	262	23	83	52	6.86	3.60	4.12
32	51	1	76	173	97	24	130	19	7.20	5.41	0.98
33	63	1	151	170	111	40	107	22	4.20	2.67	1.01
34	61	1	103	226	127	38	163	25	5.90	4.28	2.47
35	58	1	112	178	141	24	126	28	7.41	5.25	2.33
36	51	1	121	127	150	25	72	30	5.08	2.88	1.59
37	54	1	108	158	133	39	92	27	4.05	2.35	1.35
38	58	1	116	264	143	43	192	29	6.13	4.46	2.82
39	49	1	228	299	144	51	219	29	5.86	4.29	1.87
40	54	1	130	179	160	31	116	32	5.77	3.74	1.35
41	57	1	112	260	133	41	92	27	6.34	2.24	3.22
42	49	1	169	176	176	20	121	35	8.80	6.05	1.09
43	48	1	96	258	114	39	196	23	6.60	5.02	1.87
44	56	1	88	160	90	39	103	18	4.10	2.64	0.58
45	51	1	61	284	364	38	173	73	7.47	4.55	2.75
46	51	1	100	136	113	34	79	23	4.00	2.32	1.21
47	49	1	120	141	115	43	75	23	3,27	1.74	1.28
48	47	1	116	171	122	40	107	24	4.27	2.67	2.40
49	49	1	141	173	145	30	114	29	5.76	3.80	1.80
50	53	1	108	156	130	32	98	26	4.87	3.06	1.63

MASTER CHART FOR CASE GROUP

S.No	Age	Group	Glucose (mg/dL)	Total Chol (mg/dL)	Trig (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	TC/HDL Ratio	LDL/HDL Ratio	Hs- CRP (mg/L)
1	65	2	128	166	134	22	117	27	7.54	5.31	4.72
2	62	2	136	176	176	20	121	35	8.80	6.05	5.74
3	59	2	112	141	125	22	94	25	6.40	4.27	5.87
4	66	2	96	203	120	40	139	24	5.07	3.47	6.18
5	59	2	136	201	165	33	135	33	6.09	4.09	6.00
6	55	2	210	189	119	33	132	24	5.72	4.00	6.67
7	49	2	166	194	134	37	130	27	5.24	3.51	7.24
8	55	2	138	117	136	22	68	27	5.31	3.09	6.71
9	59	2	102	136	136	33	76	27	4.12	2.30	7.68
10	61	2	116	264	143	43	192	29	6.13	4.46	6.89
11	67	2	100	241	133	31	183	27	7.77	5.90	6.75
12	68	2	148	299	144	51	219	29	5.86	4.29	7.06
13	66	2	134	213	132	46	141	26	4.63	3.06	7.46
14	53	2	121	144	137	26	91	27	5.53	3.50	6.44
15	51	2	116	151	123	27	99	25	5.59	3.66	6.53
16	49	2	122	226	127	38	163	25	5.94	4.28	7.28
17	69	2	172	179	160	31	116	32	5.77	3.74	6.00
18	58	2	280	189	119	22	143	24	8.59	6.50	7.15
19	49	2	154	135	145	33	73	29	4.09	2.21	8.08
20	46	2	108	205	101	37	148	20	5.54	4.00	6.84
21	63	2	86	177	134	30	120	27	5.90	4.00	5.91
22	58	2	94	158	133	39	92	27	4.05	2.35	4.99
23	58	2	102	260	133	41	192	27	6.34	4.68	5.30
24	44	2	124	238	139	30	180	28	7.93	6.00	6.18
25	53	2	138	178	141	24	126	28	7.41	5.25	7.06
26	60	2	180	170	111	40	108	22	4.25	2.70	5.16
27	55	2	102	127	150	25	72	30	5.08	2.88	6.67
28	66	2	130	170	111	40	108	22	4.25	2.70	7.68
29	70	2	120	174	119	21	129	24	8.28	6.14	6.71
30	60	2	88	188	115	25	140	23	7.52	5.60	5.21
31	45	2	116	144	137	26	91	27	5.53	3.50	5.12
32	53	2	96	188	115	25	140	23	7.52	5.60	6.00
33	58	2	134	227	114	41	163	23	5.53	3.97	6.75
34	48	2	121	200	117	36	141	23	5.50	3.91	6.71
35	59	2	110	222	126	33	164	25	6.72	4.96	6.97
36	47	2	98	151	103	36	94	21	4.19	2.61	7.77
37	54	2	102	192	115	36	133	23	5.33	3.69	8.30
38	60	2	96	185	122	36	125	24	5.13	3.47	8.08
39	45	2	169	189	180	22	131	36	8.59	3.63	8.52
40	60	2	108	202	104	52	129	21	3.88	2.48	7.11
41	49	2	141	216	124	47	144	25	4.59	3.06	5.74
42	58	2	86	176	137	36	112	27	4.88	3.11	6.84
43	59	2	126	192	106	34	137	21	5.64	4.02	7.59
44	57	2	114	178	124	40	113	25	4.45	2.82	6.97
45	66	2	110	258	114	39	196	23	6.61	5.02	6.93
46	63	2	108	180	126	30	125	25	6.00	4.16	6.71
47	61	2	126	192	119	27	141	24	7.11	5.22	5.25
48	61	2	102	188	114	25	140	23	7.52	5.60	5.12
49	55	2	124	172	113	41	108	23	4.19	2.63	5.91
50	60	2	129	260	133	41	192	27	6.34	4.68	3.39

PROFORMA

Stanley Medical College,
Chennai.

NAME : CASE NO :

AGE/ SEX : OP/ IP NO:

H/O Presenting illness :

Past history ;

Examination :

Height : Weight

Heart rate : BP:

Respiratory rate :

Cardiovascular system : Respiratory system:

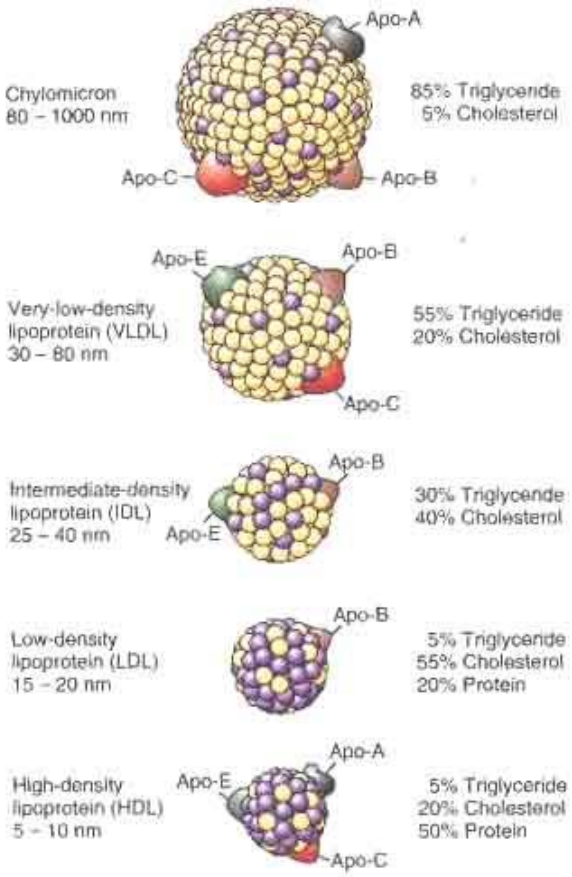
Central nervous system: Abdomen:

Investigations:

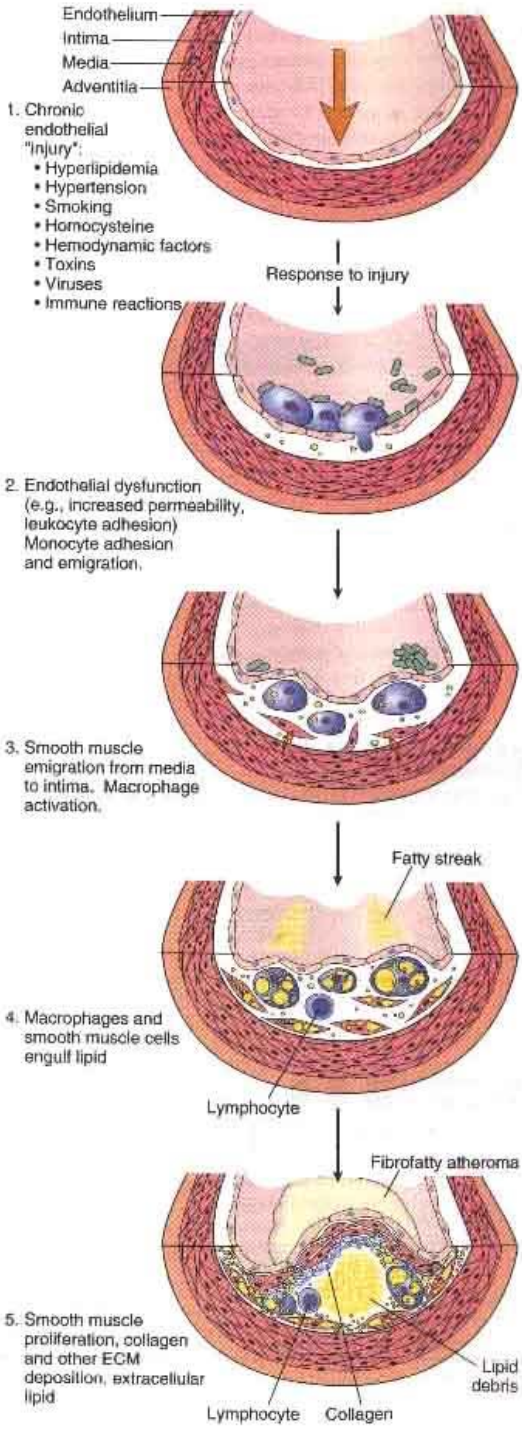
Blood Sugar:

Lipid profile:
- Total Cholesterol
- High density lipoprotein
- Triglycerides
- Calculated: Very low density lipoprotein, Low density lipoprotein.

High sensitivity C- reactive protein



Serum lipoprotein fractions showing lipid composition and apoprotein components. Binding of lipoproteins to receptors is mediated through apoproteins.



Pathogenesis of atherosclerosis. 1, chronic endothelial injury leads to 2.2,Endothelial dysfunction, permeability, and inflammation. 3, Activated monocytes infiltrate the arterial wall and smooth muscle proliferates.4, Macrophages engulf lipid to become foam cells. 5, A lipid core forms in the arterial wall and fibrous cap evolves.